

A glypican-3-derived peptide vaccine against hepatocellular carcinoma

Yu Sawada, Mayuko Sakai, Toshiaki Yoshikawa, Kazuya Ofuji and Tetsuya Nakatsura*

Division of Cancer Immunotherapy; Research Center for Innovative Oncology; National Cancer Center Hospital East; Kashiwa, Japan

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The results of a Phase I clinical trial in which a glypican-3 (GPC3)-derived peptide was tested in advanced hepatocellular carcinoma patients point to a strong correlation between immunological and clinical responses. This commentary reviews our fundamental studies and clinical trials on the GPC3-derived peptide vaccine.

The induction of tumor-specific responses in the absence autoimmunity is the ideal goal of immunotherapy. Since the identification of tumor-associated antigens in hepatocellular carcinoma (HCC), immunotherapeutic approaches have been based on the generation of tumor-specific CD8⁺ T cells that recognize peptides of 8–11 residues derived from intracellular proteins and presented in association with MHC Class I molecules.

Glypican-3 (GPC3) is a member of the glypican family of heparan sulfate proteoglycans, which are attached to the cell surface via a glycosylphosphatidylinositol (GPI) anchor. We identified GPC3 as a carcinoembryonic antigen and suggested that it would constitute an ideal target for HCC immunotherapy, due to its specific overexpression in HCC (in 81% of patients) and its correlation with poor prognosis.^{1–4} Furthermore, we identified both HLA-A24(A*2402)-restricted and H-2K^d-restricted GPC3_{298–306} (EYILSLEEL), as well as HLA-A2(A*0201)-restricted GPC3_{144–152} (FVGEFFTDV), as peptides that can induce GPC3-reactive cytotoxic T lymphocytes (CTLs) but not autoimmunity.^{2,5} HLA-A24 and A2 are the most common MHC Class I alleles in the Japanese population. By performing a binding assay, we confirmed that the HLA-A*02:01-restricted GPC3_{144–152} peptide can also bind to HLA-A*02:06 and HLA-A*02:07. We then conducted a preclinical

study in mice to design an optimal schedule for a clinical trial with the GPC3-derived peptide vaccine (Fig. 1). This study showed that incomplete Freund's adjuvant (IFA) is indispensable for GPC3 peptide-based immunotherapy, and that the immunological effects of the peptide vaccine are dose-dependent.⁶

Based on these results, we conducted a Phase I clinical trial using this GPC3-derived peptide vaccine in patients with advanced HCC, which has recently been concluded.⁷ In this study, 33 patients with advanced HCC received GPC3 peptide vaccination with dose-escalation. Peptides were emulsified with IFA and administered in liquid form by intradermal injection on days 1, 15 and 29. The GPC3_{298–306} peptide was used in HLA-A24-positive patients and the GPC3_{144–152} peptide in HLA-A2-positive patients.

In this trial, we collected evidence of immune responses, demonstrated antitumor effects, and demonstrated the safety of our GPC3-derived peptide vaccine. One patient manifested a partial response (PR) and 4 out of 19 patients with stable disease (SD) exhibit tumor necrosis or regression that did not meet the criteria for PRs. Two months after initiation of treatment, the disease control rate (PR+SD) was 60.6%. When we analyzed the frequency of GPC3-specific CTLs ex vivo by interferon γ (IFN γ) enzyme-linked immunospot (ELISPOT) assays, we could detect GPC3

peptide-specific CTLs in the peripheral blood of most patients. Alongside, we established several GPC3_{144–152} peptide-specific CTL clones from peripheral blood mononuclear cells (PBMCs) of patients vaccinated in this trial.⁸ Tumor biopsies were performed in seven patients to evaluate the infiltration of CD8⁺ T cells by immunohistochemistry. In five cases, we observed a marked intratumoral infiltration of CD8⁺ T cells upon vaccination.

A correlation between immunological and clinical responses is nowadays a required as proof for the clinical efficacy of immunotherapy. The frequency of GPC3 peptide-specific CTLs in the peripheral blood correlated with overall survival in HCC patients who received the peptide vaccination. In multivariate analysis, the frequency of GPC3-peptide-specific CTLs constitute the only predictive factor for overall survival in this trial. Analysis of all 33 patients showed a median overall survival of 12.2 mo (95% CI, 6.5–18.0) in patients with a high frequency of GPC3-specific CTLs, compared with 8.5 mo (95% CI, 3.7–13.1) in individuals with a low GPC3-specific CTL frequency ($p = 0.033$). These observations suggest that GPC3-derived peptide vaccines represent a novel immunotherapeutic strategy for patients with HCC, with a potential to improve overall survival.

We subsequently conducted a Phase II study of the GPC3-derived peptide vaccine

*Correspondence to: Tetsuya Nakatsura; Email: tnakatsu@east.ncc.go.jp
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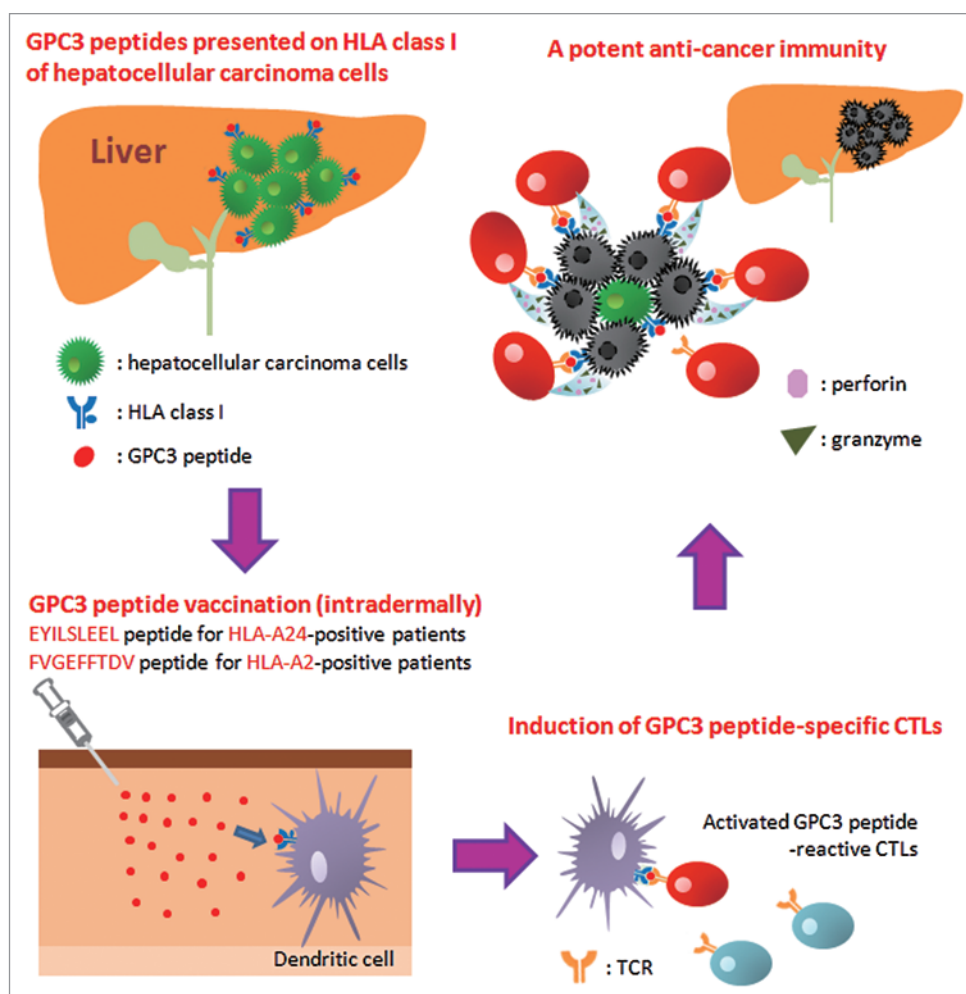


Figure 1. Mechanism of action of the GPC3-derived peptide vaccination. Most patients with hepatocellular carcinoma (HCC) exhibit an HLA-restricted glypican-3 (GPC3)-derived peptide presented in association with MHC Class I molecules. In clinical trials based on GPC3-derived peptide vaccines in HCC patients, the GPC3₂₉₈₋₃₀₆ (EYILSLEEL) peptide was used in HLA-A24-positive patients and the GPC3₁₄₄₋₁₅₂ (FVGEFFTDV) peptide in HLA-A2-positive patients. The peptides were administered with incomplete Freund's adjuvant by intradermal injection, leading to engulfment and cross-presentation by dendritic cells. Dendritic cells are capable of inducing GPC3 peptide-specific cytotoxic T lymphocytes (CTLs), which mediate anticancer immune responses.

as an adjuvant therapy for patients with HCC (UMIN-CTR: 000002614). Forty patients with HCC who had undergone surgery or radiofrequency ablation were enrolled in this Phase II, open-label, single-arm trial. Ten vaccinations were performed over 1 year after curative treatment. Primary endpoints were the 1- and 2-year recurrence rates, while secondary endpoints were immunological responses, as measured by IFN γ ELISPOT. The correlation between the time of recurrence and immunological responses is currently being analyzed.

In the Phase I trial, we did not confirm whether the tumor-infiltrating lymphocytes detected after vaccination were GPC3 peptide-specific. To address this issue, we are initiating a pilot study of liver biopsies

performed before and after GPC3 peptide vaccination for advanced HCC (UMIN-CTR: 000005093).

GPC3 is overexpressed in several malignant tumors, including ovarian clear cell carcinoma (CCC), which is normally characterized by a poor prognosis due to low sensitivity to conventional chemotherapy. We confirmed that a GPC3₁₄₄₋₁₅₂ peptide-specific CTL clone can recognize HLA-A2-positive and GPC3-positive ovarian CCC cell lines using an IFN γ ELISPOT assay, and that is can kill ovarian CCC cell lines.⁹ We are currently conducting a Phase II study with a GPC3-derived peptide vaccine in ovarian CCC patients (UMIN-CTR: 000003696).

We expect that the results of these trials will provide a rationale for larger randomized clinical trials that determine the efficacy of GPC3-derived peptide vaccines. In addition, as the antitumor effect of the peptide vaccine alone is not dramatic in advanced cancer patients, we aim to develop combinational approaches⁹ or strong antigen-specific immunotherapies, including adoptive cell transfer approaches following lymphodepletion.¹⁰ Finally, clinical trials of the adoptive cell transfer of GPC3-specific CTLs in patients with HCC in Japan are planned. Well-designed clinical trials using innovative immunotherapeutic approaches will lead to the development of efficient new therapies for the treatment of GPC3-expressing tumors.

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